(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 April 2001 (12.04.2001)

PCT

(10) International Publication Number
WO 01/25208 A1

(51) International Patent Classification[7]: C07D 213/80, 213/82, 213/87, 409/14, 409/06, A61K 31/4425, 31/4436, 31/444, A61P 37/00, 3/10

(21) International Application Number: PCT/IB99/01683

(22) International Filing Date: 15 October 1999 (15.10.1999)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
828/CAL/99 6 October 1999 (06.10.1999) IN

(71) Applicant and
(72) Inventor: SANKARANARAYANAN, Alangudi [IN/IN]; B-7, Swastik Park, Premchandnagar Road, Bodakdev, Ahmedabad 380054, Gujarat (IN).

(74) Agents: AHUJA, Sudhir, D. et al.; D. P. Ahuja & Co., 53, Syed Amir Ali Avenue, Calcutta 700019, West Bengal (IN).

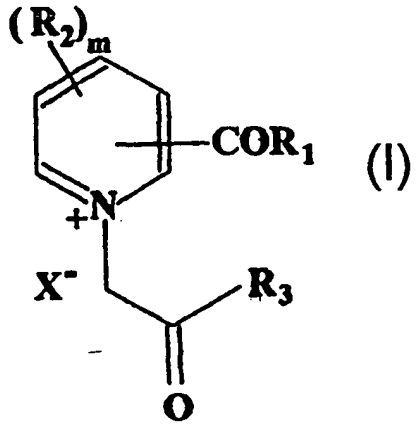
(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PYRIDINIUM DERIVATIVES FOR THE MANAGEMENT OF AGING-RELATED AND DIABETIC VASCULAR COMPLICATIONS, PROCESS FOR THEIR PREPARATION AND THERAPEUTIC USES THEREOF



(I)

(57) Abstract: The invention discloses novel compounds of the pyridinium series useful for the management of diabetes and aging-related vascular complications, including kidney disease, nerve damage, atherosclerosis, retinopathy, dermatological disorders and discoloration of teeth, by breaking preformed AGE, of general formula (I), or pharmaceutically acceptable salts thereof, wherein, R_1, R_2, R_3, X and m are as defined in the specification. The invention also discloses, method for preparation of the novel compounds of the series and pharmaceutical composition having one or more compounds as defined above as active ingredients. The invention further discloses a method of treatment of a diabetic patient by administering the compounds as defined above, either singly or in combination with drugs for antidiabetic therapy.

WO 01/25208 A1

Title: PYRIDINIUM DERIVATIVES FOR THE MANAGEMENT OF AGING-RELATED AND DIABETIC VASCULAR COMPLICATIONS, PROCESS FOR THEIR PREPARATION AND THERAPEUTIC USES THEREOF

FIELD OF THE INVENTION

The present invention relates to a new class of compounds of pyridinium series and to their use in treatment of diabetes and related illnesses. More particularly the invention relates to compounds of this series, methods for their preparation, pharmaceutical composition containing these compounds and their use in the treatment of complications of diabetes mellitus. The compounds of this series exhibit AGE breaking activity, which is essential for the treatment of diabetic and aging-related complications including kidney disease, nerve damage, atherosclerosis, retinopathy and dermatological conditions. The invention also extends to the method of reversing the discoloration of teeth resulting from nonenzymatic browning in the oral cavity which comprises administration of an amount effective to reverse pre-formed advanced glycosylation crosslinks.

BACKGROUND OF THE INVENTION

Maillard in 1912 found that reducing sugars, such as glucose and ribose react with proteins to form brown pigments. Further studies have shown that this

is an irreversible non-enzymatic reaction, which occurs in several natural systems including stored foodstuff. Maillard reaction occurs in two stages, early and advanced. Initially, proteins react with glucose to form stable Amadori products, which subsequently cross-links to form advanced glycation end products (AGE). In most cases, the formation of AGE also accompanies browning of the proteins and increase in the fluorescence.

In diabetes, where blood glucose level is significantly higher than normal, the reaction of glucose with several proteins such as haemoglobin, lens crystallin and collagen, gives rise to the formation of AGE, which in turn, is responsible for the complications associated with diabetes, such as nephropathy, microangiopathy, endothelial dysfunction and other organ dysfunctions. In addition, the activity of several growth factors, such as basic fibroblast growth factor, is also impaired. AGE products, unlike normal proteins in tissue, have a slower rate of turnover and replenishment. It has been reported that AGE products may in fact elicit a complex immunological reaction involving RAGE (Receptor for Advanced Glycation End Products) receptors and activation of several incompletely defined immunological processes. It has been documented that diabetes with evidence of microangiopathy and macroangiopathy also show evidence of oxidative stress, the mechanism of which has not been elucidated.

In vitro AGE formation can be studied in the laboratory by incubating reducing sugars, such as ribose or glucose with bovine serum albumin. AGE formation can be detected by increase in the fluorescence or increased cross reactivity with anti-AGE antibodies. The increase in fluorescence seems to precede formation of AGE specific antigenic epitopes. This increase in fluorescence is used to monitor the increased AGE formation in vitro (Brownlee M et al, Science 1986; 232:1629-1632). In addition to the increase in the fluorescence, one of the most important features of in vitro AGE formation is the formation of antigenic epitopes that are specific to AGE and not to the native proteins. Therefore, it is possible to raise antibodies against advanced glycation end products of one protein and use them to detect AGE formation in other proteins. This has served as an important analytical tool in AGE research.

Due to the clinical significance of AGE formation, many approaches are being used to diagnose, prevent, or revert AGE formation in the body. The formation of AGE could be inhibited by reacting with an early glycosylation product that results from the original reaction between the target protein and glucose. The inhibition was believed to take place as the reaction between the inhibitor and the early glycosylation product appeared to interrupt the subsequent reaction of the glycosylated protein with additional protein material to form the

cross linked late stage product. Compounds like aminoguanidine act to inhibit AGE formation by such mechanism.

The formation of AGE on long-lived proteins is also associated with cross-linking of these proteins. The AGE derived protein cross-links have been shown to be cleaved by compounds like N- phenacyl thiazolium bromide (PTB), which reacts with and cleaves covalent, AGE derived protein cross links (Vasan et al. Nature 1996; 382: 275-278 ; US 5,853,703, Date of Patent : Dec. 29, 1998). The mechanism of reducing the AGE content in tissues is expected to take place relatively rapidly, in contrast to aminoguanidine, which acts slowly by its very nature of mechanism of action. This current specification is related to compounds of pyridinium class, which break pre-formed AGE, like PTB, and in some cases even more effectively by than PTB.

SUMMARY OF THE INVENTION

The main objective of the present invention is to provide a new class of compounds of the pyridinium series which are useful for the management of diabetes and aging related vascular complications and particularly in the treatment of complications of diabetes mellitus and other aging related conditions including kidney disease, nerve damage, atherosclerosis, retinopathy and dermatological conditions. The invention also extends the method to reverse the discoloration of

teeth resulting from nonenzymatic browning in the oral cavity which comprises administration of an amount effective to reverse the pre-formed advanced glycosylation crosslinks, etc.

Another object of the present invention is to provide compounds of the pyridinium series, which exhibit AGE breaking activities.

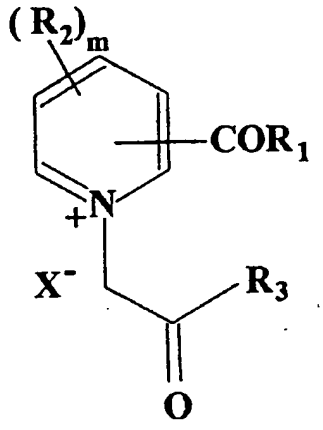
Yet another object of the present invention is to provide a method of preparation of compounds of the pyridinium series which exhibit AGE breaking activities.

Still another object of the invention is to provide pharmaceutical compositions with a new class of compounds of the pyridinium series according to the invention and their pharmaceutically acceptable salts in combination with suitable carriers, solvents, excepients, diluents and other media normally employed in preparing such compositions.

Still another object of the invention is to provide a method of treatment of a diabetic patient by administration of the compounds of the invention, either singly or in combination with drugs for anti-diabetic therapy, or pharmaceutically acceptable salts thereof in required dosage in admixture with pharmaceutically acceptable diluent, solvent, excepients, carriers or other media as may be appropriate for the purpose.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for a new class of AGE breakers, of general formula I,



(I)

wherein

R_1 is $-R_4-R_5$ or $-N(R_7)\,N\,(R_7)\,R_9$;

R_4 is selected from the group $-N(R_7)R_6O-$, $-N(R_7)R_6N(R_7)-$, OR_6O, $-OR_6N(R_7)-$, where R_6 is alkyl;

R_5 is selected from the group alkyl, aryl, including heteroaryl, $-COR_7$, SO_2R_7, $-C(S)\,NHR_7$, $-C(NH)NHR_7$, $-COR_{10}$,

$-C(O)NHR_7$ and $-N(R_7)\,N{=}C(R_{10})(R_7)$

where R_7 is selected from the group H, alkyl or aryl, including heteroaryl;

R_2 is selected from the group F, Cl, Br, I, OR_7, NO_2, alkyl, aryl including heteroaryl, formyl, acyl, $C(O)NR_7R_{10}$, $C(O)OR_7$, NR_7R_{10}, $N=C(R_7)(R_{10})$, SR_7, SO_2NH_2, SO_2 alkyl and SO_2aryl,

and m is 0, 1 or 2

R_3 is selected from the group R_7, OR_7, $N(R_7)(R_{10})$, $N=C(R_7)(R_{10})$, $N(R_7)N(R_7)(R_{10})$, $N(R_7)N=C(R_7)(R_{10})$ and $CH(R_7)C(O)R_8$

where R_8 is selected from the group R_7, OR_7 and NR_7R_{10};

R_9 is selected from the group consisting of hydrogen, alkyl, aryl, including heteroaryl, $C(O)R_{10}$, $-SO_2R_{10}$, $-C(S)NHR_{10}$, $-C(NH)NH(R_{10})$, $-C(O)NHR_{10}$,

R_{10} is selected for the group H, alkyl or aryl, including heteroaryl and in each case optionally different from substituent R_7

X is selected from group consisting of a halide ion, acetate ion, perchlorate ion, sulfonate ion, oxalate ion, citrate ion, tosylate ion, maleate ion, mesylate ion, carbonate ion, sulfite ion, phosphoric hydrogen ion, phosphonate ion, phosphate ion, BF_4^-, PF_6^-, etc.

with proviso that

(i) when two alkyl groups are present on the same carbon or nitrogen, they are optionally linked together to form a cyclic structure and

(ii) the nitrogen of heteroaryl ring of R_{10}, when present, is optionally quaternized with compound such as $X\text{-}CH_2C(O)\text{-}R_3$

As used herein, "alkyl" refers to an optionally substituted hydrocarbon group joined by single carbon-carbon bonds and having 1 to 8 carbon atoms joined together. The alkyl hydrocarbon group may be linear, branched or cyclic, saturated or unsaturated. The substituents are selected from F, Cl, Br, I, N, S, O and aryl. Preferably, no more than three substituents are present.

As used herein "aryl" refers to an optionally substituted aromatic group with atleast one ring having a conjugated pi- electron system, containing upto two conjugated or fused ring systems. Aryl includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The substituents are selected from F, Cl, Br, I, N, S, O and straight chain or branched C_1-C_6 hydrocarbon.

The novel compounds of the invention of general formula I having m as 0 and - COR_1 at position 3 are listed in Table 1A and the novel compounds of the invention of general formula I having m as 0 and - COR_1 at position 4 are listed in Table 1B. The following compounds suggested are by way of example alone of the representative compounds of the general formula I as defined above and in no way restrict the invention.

N,N'-Bis[3-carbonyl-1-(2-phenyl-2-oxoethyl)-pyridinium] hydrazine dibromide (compound 1):

N,N'-Bis[3-carbonyl-1-(2-ethoxy -2- oxoethyl)pyridinium]hydrazine dibromide (compound 2):

N,N'-Bis[3-carbonyl-1-(2-(2,4-dichlorophenyl)-2-oxoethyl)pyridinium]hydrazine dibromide (compound 3):

1- (2- Ethoxy -2- oxoethyl) -3- (2- (2- pyridyl) hydrazinocarbonyl) pyridinium bromide (compound 4):

1- (2- Thien -2'- yl -2- oxoethyl) -3- (methanesulfonyl hydrazinocarbonyl) pyridinium bromide (compound 5):

N,N'-Bis[3-carbonyl-1- (2- thien -2'- yl -2- oxoethyl)pyridinium]hydrazine dibromide (compound 6):

1- (2- Ethoxy -2- oxoethyl) -3- (2- (benzoyloxy) ethylaminocarbonyl) pyridinium bromide (compound 7):

1- (2- (2,4- Dichlorophenyl) -2- oxoethyl) -3- (2-(benzoyloxy)ethylamino-carbonyl) pyridinium bromide (compound 8):

1- (2- Thien -2'- yl -2- oxoethyl) -3- (2- (2- pyridyl) hydrazinocarbonyl) pyridinium bromide (compound 9):

1- (2- Phenyl -2- oxoethyl) -3- (2- (2- pyridyl)hydrazinocarbonyl) pyridinium bromide (compound 10):

1-(2-Phenyl-2-oxoethyl)-3-(hydrazinocarbonyl)pyridinium bromide (compound 11).

1-(2- Phenyl -2- oxoethyl) -3- (methanesulfonyl hydrazinocarbonyl) pyridinium bromide (compound 12):

1- (2- Ethoxy -2- oxoethyl) -3- (methanesulfonyl hydrazinocarbonyl) pyridinium bromide (compound 13):

1-(2-Phenyl-2-oxoethyl) -3- (phenylsulfonylhydrazino carbonyl) pyridinium bromide (compound 14):

1-(2-Phenyl-2-oxoethyl) -2-chloro-3- (phenylsulfonylhydrazino carbonyl) pyridinium bromide (compound 15):

1-(2- Phenyl -2- oxoethyl) -3- (2- (methoxy)carbonyl)ethyloxy carbonyl pyridinium bromide (compound 16):

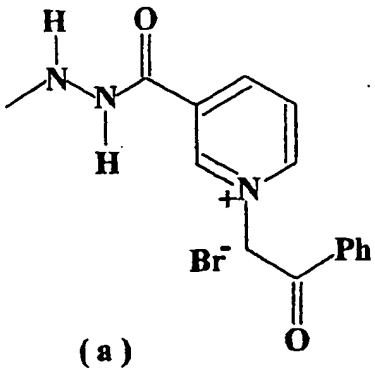
1-(2-Ethoxy -2- oxoethyl) -3- (2- (benzoyloxy) ethyloxy carbonyl) pyridinium bromide (compound 17):

1-(2- Thien -2'- yl -2- oxoethyl)-4-(2-(benzoyloxy)ethylaminocarbonyl) pyridinium bromide (compound 18):

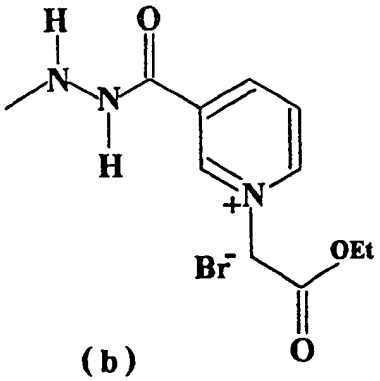
Table 1A – Representative Pyridinium derivatives

(having m as 0 and -COR_1 at position 3)

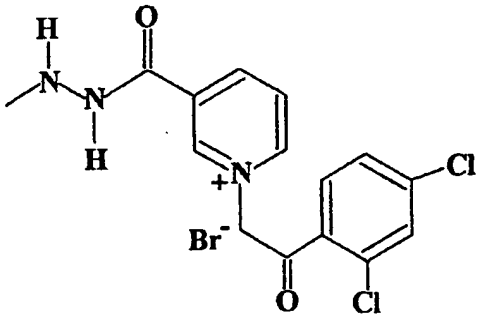
Compound	-R_1	-R_2	-R_3	-X
1	Structure(a)	-	phenyl	Br
2	Structure (b)	-	OEt	Br
3	Structure (c)	-	2,4-dichlorophenyl	Br
4	NHNH-(2-pyridyl)	-	OEt	Br
5	$NHNHSO_2CH_3$	-	2-thienyl	Br
6	Structure (d)	-	2-thienyl	Br
7	$NHCH_2CH_2OCOPh$	-	OEt	Br
8	$NHCH_2CH_2OCOPh$	-	2,4-dichlorophenyl	Br
9	NHNH-(2-pyridyl)	-	2-thienyl	Br
10	NHNH-(2-pyridyl)	-	phenyl	Br
11	$NHNH_2$	-	phenyl	Br
12	$NHNHSO_2CH_3$	-	phenyl	Br
13	$NHNHSO_2CH_3$	-	OEt	Br
14	NHNH-SO_2phenyl	-	phenyl	Br
15	NHNH-SO_2phenyl	2-Cl	phenyl	Br
16	$OCH_2CH_2OCOCH_3$	-	phenyl	Br
17	OCH_2CH_2OCOPh	-	OEt	Br



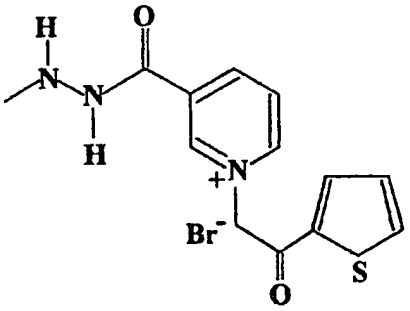
(a)



(b)



(c)



(d)

Table 1B – Representative Pyridinium derivatives

(having m as 0 and -COR_1 at position 4)

Compound	-R_1	-R_2	-R_3	-X
18	$NHCH_2CH_2OCOPh$	-	2-thienyl	Br

According to the embodiment of the present invention, the present compounds are used for the treatment of diabetic complications, and aging related

complications including kidney disease, nerve damage, atherosclerosis, retinopathy, dermatological conditions and colouration of teeth occurring due to the higher levels of preformed AGE. The increased levels of preformed AGE can be brought under control by breaking the AGE products using compounds mentioned in the invention.

The invention also provides a process for the preparation of novel compounds of the pyridinium series.

The said process for the preparation of compound 1, comprises, adding a solution of phenacyl bromide in isopropanol to N,N'-bis-(nicotinyl)hydrazine dissolved in methanol, refluxing for six hours, cooling, filtering the precipitated solid, washing the solid with hot ethyl acetate and finally purifying the solid with 20 ml of methanol : ethyl acetate (3 : 1) to yield the desired compound.

Similarly, the other novel compounds of general formula I, are prepared from properly substituted pyridine derivatives followed by quarternization with appropriate reagent by refluxing in alcoholic solvents like, methanol, ethanol, propanol, etc and high boiling solvents like toluene or xylene etc, for 6 - 48 hrs. to give the desired compounds.

The in vitro AGE formation, studied in the laboratory, by incubating reducing sugar ribose, with protein bovine serum albumin, resulted in browning of

solution and increase in the fluorescence. Fluorescence was used as the criteria to monitor the increased AGE formation.

Example 1

AGE breaker activity has been confirmed by the screening procedure as mentioned below:

Materials:

Bovine serum albumin (fraction V) (BSA)

Ribose, analytical grade

Phosphate buffered saline (PBS)

Equipment:

Microplate ELISA Reader - Spectramax Plus (Molecular Devices, USA)

Microplate washer, (Bio -Tec Instruments, USA)

pH meter

Methods of experiment:

160 mg/ml of protein, bovine serum albumin, BSA and 1.6M glucose sugar were dissolved in phosphate buffered saline, PBS. Sodium azide was added at 0.02% concentration as a preservative. The solution was filtered asceptically through a 0.22 μM filter and kept for aging at 37°C for 16 weeks. After 16 weeks the solution was dialyzed against PBS, aliquoted and stored at - 20°C.

To determine the AGE breaking activity, 10μg/ml and 100μg/ml of the 16 weeks AGE-BSA was incubated with different concentrations of the test compounds at 37°C for 24 hours and AGE breaking activity of the test compounds by ELISA was determined.

ELISA was performed as follows:

1. Different concentrations of 16 weeks AGE-BSA were coated on a microtitre plate as standard. Each concentration is coated in triplicates.

2. The test samples were coated on microtitre plate at a concentration of 5 ng. to 20 ng per well in triplicates.

3. The plate was incubated at 37°C for one hour.

4. After incubation the plate was washed with PBST (PBS with 0.05% Tween 20).

5. Blocking with 5% skimmed milk in PBS at 37°C for one hour was done.

6. The plate was washed with PBST.

7. Primary antibody against AGE-BSA was added and the plate is incubated at 37°C for one hour.

8. The plate was washed with PBST

9. Secondary antibody anti rabbit HRPO (Horse-Radish Per Oxidase) conjugate was added and the plate is incubated at 37°C for one hour.

10. The plate was washed with PBST.

11. Colour development with OPD (orthophenylenediamine dihydrochloride) and hydrogen peroxide was done.

12. OD (optical density) at (450nm reading - 620nm reading) was measured after incubation at 37°C for 15 minutes with Microplate ELISA Reader.

The breaker activity of the compounds were determined by the following formula:

$$\% \text{ Breaker activity} = \frac{OD_{450\text{-}620}\text{Control} - OD_{450\text{-}620}\text{Test}}{OD_{450\text{-}620}\text{Control}} \times 100$$

$OD_{450\text{-}620}$Control= Absorbance of 20ng AGE-BSA after incubation at 37°C for 24 hours without test compound

$OD_{450\text{-}620}$Test= Absorbance of 20ng AGE-BSA after incubation at 37°C for 24 hours with required concentration of test compound

Using specific examples, the % AGE breaking activity was calculated and recorded in Table 2.

Table 2

Sample	Concentration	% Breakage
PTB	10 mM	27
	20 mM	47
Compound 1	5 mM	13
Compound 4	10 mM	30

Compound 6	5 mM	53
Compound 7	20 mM	36
Compound 16	10 mM	16
Compound 17	10 mM	19

Hence compound 6 has significant AGE breaking activity i.e. a comparatively much superior potency vis - a - vis PTB.

The following examples give method of preparation of the specific novel compounds of the invention as given in Table 1. The following compounds suggested are by way of example alone and in no way restrict the invention.

Example 2

Preparation of N,N'-bis [3-carbonyl-1- (2-phenyl-2-oxoethyl) pyridinium] hydrazine dibromide (compound 1):

To a boiling solution of N,N'-bis-(nicotinyl)hydrazine (1.21 g., 0.005 mol.) in methanol (20 ml.), a solution of phenacyl bromide (1.99 g., 0.01 mol.) in isopropanol (10 ml.) was added and the reaction mixture was refluxed for 6 hrs. The reaction mixture was concentrated under vacuum (~10 ml.) and filtered. The obtained residue was washed with hot ethylacetate and then the isolated solid was powdered. It was recrystallised from a mixture of methanol and ethylacetate (3:1,

20 ml) to afford a pale yellow solid.

Yield : 60%

m.p. : 260 - 262°C (decomp.)

IR(KBr, cm^{-1}) : 1696 and 1680

^1H NMR ($DMSOd_6$, 400MHz) δ: 11.65(2H,s), 9.56(2H,s), 9.21-9.16(4H,m), 8.49-8.45 (2H,m), 8.08-8.05 (4H,d), 7.81-7.77(2H,m), 7.68-7.64 (4H,m), 6.58 (4H,s)

Mass (m/z) : 479, 480

According to the above mentioned procedure the following compounds are synthesized by reacting the corresponding pyridine derivatives with appropriate reagents by refluxing in methanol, ethanol, propanol, toluene or xylene for 6 - 48 hrs. to get the desired compounds:

Example 3

N,N'-Bis[3-carbonyl-1- (2- ethoxy -2-oxoethyl) pyridinium] hydrazine dibromide (compound 2):

Yield : 47%

m.p. : 180 - 182°C (decomp.)

IR(KBr, cm^{-1}) : 1744, 1664

^1H NMR ($DMSOd_6$, 400MHz) δ: 11.65 (2H,s), 9.62 (2H,s), 9.28-9.26 (2H,d), 9.17-9.15 (2H,d), 8.47-8.44 (2H,m), 5.77 (4H,s), 4.26 (4H,q), 1.27 (6H,t)

Mass (m/z) : 415, 416

Example 4

N,N'-Bis[3-carbonyl-1- (2- (2,4- dichlorophenyl) -2- oxoethyl) pyridinium] hydrazine dibromide (compound 3):

Yield : 24%

m.p. : 225 - 227°C (decomp.)

IR(KBr, cm^{-1}) : 1702, 1666

^1H NMR ($DMSOd_6$, 400 MHz) δ: 11.69 (2H,s), 9.58 (2H,bs), 9.20-9.18 (4H,m), 8.49-8.47 (2H,m), 8.17-8.15 (2H,d), 7.92 (2H,bs), 7.78-7.76 (2H,d), 6.50 (4H,s)

Mass (m/z) : 615, 617, 618, 620.

Example 5

1- (2- Ethoxy -2- oxoethyl) -3- (2- (2- pyridyl) hydrazinocarbonyl) pyridinium bromide (compound 4):

Yield : 16%

m.p. : 210-212°C

IR (KBr, cm^{-1}) : 3140, 3005, 1732 and 1690

^1H NMR ($DMSOd_6$, 400MHz) δ: 9.63 (1H,s), 9.27 (2H,d), 8.49-8.45 (1H,m) 8.13-8.07 (2H,m), 7.32-7.30 (1H,m), 7.12-7.11(1H,m), 5.77 (2H,s), 4.23 (2H,q), 1.25 (3H,t)

Mass (m/z) : 301, 302

Example 6

1- (2- Thien -2'- yl -2- oxoethyl) -3- (methanesulfonyl hydrazinocarbonyl) pyridinium bromide (compound 5):

Yield : 30 %

m.p : 199 – 200 °C

IR (KBr, cm^{-1}): 1714, 1673

^{1}HNMR ($DMSOd_6$, 400 MHz) δ: 11.38 (1H,s), 9.97 (1H,s) 9.51 (1H,s), 9.16 (1H,d), 9.06 – 9.04 (1H,m), 8.43 – 8.39 (1H,m), 8.25 – 8.21 (2H,m), 7.43 – 7.41 (1H,t), 6.45 (2H,s), 3.08 (3H,s).

Mass (m/z) : 340, 341, 342

Example 7

N,N'-Bis[3-carbonyl-1- (2- thien -2'- yl -2- oxoethyl)pyridinium]hydrazine dibromide (compound 6):

Yield : 33%

m.p. : 259 - 261°C (decomp.)

IR (KBr, cm^{-1}) : 3330, 1702, 1674, 1655 and 1626

^{1}H NMR ($DMSOd_6$, 400 MHz) δ: 11.59 (2H,s), 9.50 (2H,s), 9.15-9.08 (4H,m), 8.40-8.36 (2H,m), 8.17-8.14 (4H,m), 7.33(2H,t), 6.42 (4H,s)

Mass (m/z) : 491, 492.

Example 8

1- (2- Ethoxy -2- oxoethyl) -3- (2- (benzoyloxy) ethylaminocarbonyl) pyridinium bromide (compound 7):

Yield : 85%

m.p. : 132-134°C

IR (KBr, cm^{-1}) : 3210, 3067, 1726, 1687, 1656

^1H NMR ($DMSOd_6$, 400 MHz) δ : 9.46 (1H,s), 9.37 (1H,t), 9.11(1H,t), 8.97 (1H,d), 8.33-8.29 (1H,m) 7.95-7.93 (2H,m), 7.63-7.59 (1H,m), 7.49-7.45 (2H,m), 5.65 (2H,s), 4.39 (2H,t), 4.19 (2H,q), 3.70-3.69 (2H,m), 1.20 (3H,t)

Mass (m/z) : 357, 358, 359

Example 9

1- (2- (2,4- Dichlorophenyl) -2- oxoethyl) -3- (2-(benzoyloxy)ethyl aminocarbonyl) pyridinium bromide (compound 8):

Yield : 75%

m.p. : 102-104°C

IR(KBr, cm^{-1}): 1703, 1685, 1675

^1H NMR ($DMSOd_6$, 400 MHz) δ: 9.41-9.37 (2H,m), 9.03-8.98 (2H,m)8.34-8.30 (1H,m), 8.04 (1H,d), 7.91-7.89 (2H,m), 7.82 (1H,d),7.68-7.65 (1H,m), 7.58-7.55 (1H,m), 7.43 (2H,t), 6.35 (2H,s), 4.36 (2H,t), 3.68-3.64 (2H,m)

Mass (m/z) : 457, 458, 459, 460, 461, 462

Example 10

1- (2- Thien -2'- yl -2- oxoethyl) -3- (2- (2- pyridyl) hydrazinocarbonyl) pyridinium bromide (compound 9):

Yield : 10%

m.p. : 212-214°C (decomp)

IR(KBr, cm^{-1}) : 1685, 1649

^1H NMR ($DMSOd_6$, 400 MHz) δ: 11.21 (1H,bs), 9.59 (1H,s), 9.19 (2H,d), 8.44 (1H,t), 8.27-8.24 (2H,m), 8.08 (1H,bs), 7.62 (1H,bs), 7.44 (1H,t), 6.85-6.79 (2H,m), 6.50 (2H,s)

Mass (m/z) : 339, 340, 341

Example 11

1- (2- Phenyl -2- oxoethyl) -3- (2- (2- pyridyl) hydrazinocarbonyl) pyridinium bromide (compound 10):

Yield : 4%

m.p. : 190°C (decomp)

IR(KBr, cm^{-1}) : 1683, 1670, 1648

^1H NMR ($DMSOd_6$, 400 MHz) δ: 11.14 (1H,bs), 9.53 (1H,s), 9.18-9.13 (2H,m), 8.45-8.42 (1H,t), 8.08-8.06 (3H,m), 7.80 (1H,t), 7.67 (2H,t), 7.62-7.55 (1H,m), 6.83-6.76 (2H,m), 6.54 (2H,s)

Mass (m/z) : 333, 334, 335

Example 12

1-(2-Phenyl-2-oxoethyl) -3- (hydrazinocarbonyl) pyridinium bromide (compound 11).

Yield : 15%

m.p. : 215 – 216 °C

IR(KBr, cm^{-1}) : 1695, 1680

^{1}HNMR ($DMSOd_6$, 400 MHz) δ : 10.25 (1H,s) 9.65 (1H,s), 9.35 – 9.32 (2H,m), 8.90 – 8.88 (1H,m) 8.50 – 8.46 (2H,d), 8.21 – 8.17 (1H,m), 8.05 – 8.07 (2H,m), 6.50 (2H,s), 4.45 (2H,s).

Mass (m/z) : 256, 257.

Example 13

1- (2- Phenyl -2- oxoethyl) -3- (methanesulfonyl hydrazinocarbonyl) pyridinium bromide (compound 12):

Yield : 35%

m.p.: 227 – 228 °C

IR(KBr, cm^{-1}): 1710, 1702

^{1}HNMR ($DMSOd_6$, 400 MHz) δ : 11.30, (1H,s), 9.88 (1H,s), 9.41 (1H,s), 9.06 – 9.05 (1H,d) 8.98 – 8.96 (1H,d), 8.34 – 8.31 (1H,m), 7.97 (2H,d), 7.72 – 7.69 (1H,t), 7.59 – 7.56 (2H,t), 6.44 (2H,s), 2.99 (3H,s)

Mass (m/z): 334, 335

Example 14

1-(2- Ethoxy -2- oxoethyl) -3- (methanesulfonyl hydrazinocarbonyl) pyridinium bromide (compound 13):

Yield : 38%

m.p: 75- 76 °C

IR(KBr, cm^{-1}): 1739, 1697

^{1}HNMR ($DMSOd_6$, 400 MHz) δ : 11.39 (1H,s), 9.96 (1H,s), 9.56 (1H,s), 9.23 (1H,d), 9.06 (1H,d), 8.40 (1H,t), 5.75 (2H,s), 4.27 – 4.22 (2H,q), 3.08 (3H,s), 1.26 (3H,t)

Mass (m/z): 301, 302, 303

Example 15

1-(2-Phenyl-2-oxoethyl)-3-(phenylsulfonylhydrazino carbonyl) pyridinium bromide (compound 14):

Yield : 28%

m.p: 218 - 219°C

IR(KBr, cm^{-1}): 1687 , 1677

^{1}HNMR ($DMSOd_6$, 400 MHz) δ : 11.01 (1H,s), 9.53 (1H,s), 9.17 – 9.16 (2H,m), 8.44 (1H, t), 8.07 (2H,d), 7.80 (1H,t), 7.67 (2H,t), 7.18 (2H,t), 6.87 (2H,d), 6.77 (1H,t), 6.56 (2H,s).

Mass (m/z) : 461, 462

Example 16

1- (2-Phenyl-2-oxoethyl)-2-chloro-3-(phenylhydrazino carbonyl) pyridinium bromide (compound 15):

Yield : 23%

m.p. : 247 - 250°C (decomp)

IR(KBr, cm^{-1}): 1685 , 1679,

^{1}HNMR ($DMSOd_6$, 400 MHz) δ : 11.12 (1H,s), 9.49 (1H,s), 9.07 – 9.03(1H,m), 8.44 (1H, t), 8.07 (2H,d), 7.80 (1H,t), 7.67 (2H,t), 7.18 (2H,t), 6.87 (2H,d), 6.77 (1H,t), 6.50 (2H,s).

Mass (m/z) : 366, 367, 368

Example 17

1-(2- Phenyl -2- oxoethyl) -3- (2- (methoxycarbonyl) ethyloxy carbonyl pyridinium bromide (compound 16):

Yield : 40%

m.p. : 134-136°C

IR(KBr, cm^{-1}) :1710, 1670, 1668

^{1}HNMR ($DMSOd_6$, 400 MHz) δ :9.57(1H,s), 9.14-9.08(2H,m), 8.37-8.34(1H,m), 8.00-7.98(2H,d), 7.74-7.70(1H,t), 7.61-7.57(2H,t), 6.49(2H,s), 4.36-4.33(2H,t), 3.67-3.65(2H,t), 1.99(3H,s)

Mass (m/z) :328, 329, 330

Example 18

1- (2- Ethoxy -2- oxoethyl) -3- (2- (benzoyloxy) ethyloxycarbonyl) pyridinium bromide (compound 17):

Yield : 35%

m.p. : 142-143°C

IR(KBr, cm^{-1}) : 1728, 1685, 1660

^{1}HNMR ($DMSOd_6$, 400 MHz) δ : 9.60(1H,s), 9.20-9.18(1H,d), 7.04-9.02(1H,d), 8.33-8.29(1H,m), 7.90-7.88(2H,d), 7.58-7.57(1H,m), 7.46-7.42(2H,m), 5.67(2H,s), 4.71-4.68(2H,m), 4.58-4.56(2H,m), 4.15(2H,q), 1.16(3H,t)

Mass (m/z) : 358, 359, 360

Example 19

1- (2- Thien -2'- yl -2- oxoethyl)-4-(2-(benzoyloxy)ethylaminocarbonyl) pyridinium bromide (compound 18):

m.p. : 210 – 211°C

IR(KBr, cm^{-1}) : 1723, 1680, 1668

^{1}HNMR ($DMSOd_6$, 400 MHz) δ : 9.52 (1H,t), 9.14 (2H,d), 8.50 (2H,d), 8.25 – 8.21 (2H,m), 8.01 – 7.99 (2H,d), 7.67 (1H,t), 7.55 – 7.51 (2H,m), 7.42 – 7.40 (1H,m), 6.42 (1H,s) 4.47 – 4.45 (2H,t), 3.77 – 3.73 (2H, m).

Mass (m/z) : 395, 396

Pharmaceutical Compositions

Pharmaceutical compositions may be prepared with a pharmaceutically effective quantity of compounds of general formula I, individually or in combination. The following pharmaceutical formulations suggested are by way of example alone and in no way restrict the forms in which they can be used.

Oral formulations

Oral formulations may be administered as solid dosage forms for example pellets, powders, sachets or discreet units such as tablets or capsules and like. Other orally administered pharmaceutical preparations include monophasic and biphasic liquid dosage forms either in ready to use form or forms suitable for reconstitution such as mixtures, syrups, suspensions or emulsions. The preparations in addition may contain diluents, dispersing agents, buffers, stabilizers, solubilizers, surfactants, preservatives, chelating agents and/ or other pharmaceutical additives as are used. Aqueous or non aqueous vehicle or their combination may be used and if desired may contain suitable sweetener, flavoring agent or similar substances. In case of suspension or emulsion a suitable thickening agent or suspending agent or emulsifying agent may be present in addition. Alternatively, the compounds may be administered as such in their pure form unassociated with other additives for example as capsules or sachets. It may also be administered with a vehicle. Pharmaceutical preparations can have a slow,

delayed or controlled release of active ingredients as is provided by a matrix or diffusion controlled system.

When the present invention or its salts or suitable complexes is presented as a discreet unit dosage form like tablet, it may contain in addition medically inert excipients as are used in the art. Diluents such as starch, lactose, dicalcium phosphate, talc, magnesium stearate, polymeric substances like methyl cellulose, fatty acids and derivatives, sodium starch glycollate, etc. may also be used.

Example 20

Preparation of oral dosage form:

A typical tablet has the following composition:

Active ingredient of formula I	as given above
Lactose	135 mg
Starch	76 mg
Polyvinyl pyrolidone (K-30)	2 mg
Talc	1.5 mg
Magnesium Stearate	1.0 mg

Parenteral Formulations

For parenteral administration, the compounds or their salts or suitable complexes thereof may be present in a sterile vehicle which may be an aqueous or non aqueous vehicle or a combination thereof. The examples of vehicles are

water, ethyl oleate, oils and derivatives of polyols, glycols and their derivatives. It may contain additives common in injectable preparations like stabilizers, solubilizers, pH modifiers, buffers, antioxidants, cosolvents, complexing agents, tonicity modifiers, etc.

Some suitable additives are for example tartrate, citrate or similar buffers, alcohol, sodium chloride, dextrose and high molecular weight polymers. Another alternative is sterile powder reconstitution. The compound may be administered in the form of injection for more than once daily administration, or intravenous infusion/ drip or suitable depot preparation.

Example 21

Preparation suitable for parenteral administration has the following composition:

Active ingredient of formula I	as given above
Polyethylene glycol (400)	0.75 ml
Sodium metabisulphite	0.01%
Isotonic saline/ WFI	q.s.

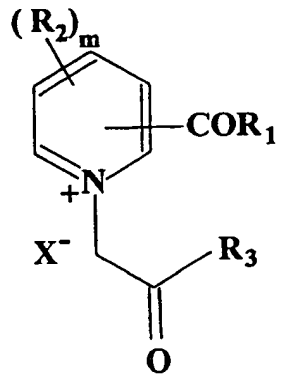
Other Formulations.

For the dermatological application and for the discoloration of teeth, the recommended formulations are lotions, oral rinse and toothpaste containing appropriate amount of the compounds of the general formula I.

The above examples are presented by way of illustration alone and in no way limit the scope of the invention.

I CLAIM:

1. A compound of pyridinium series of general formula I, and its pharmaceutically acceptable salts, useful for the management of vascular complications associated with diabetes and aging related disorders,



(I)

wherein

R_1 is $-R_4-R_5$ or $-N(R_7)\,N\,(R_7)\,R_9$;

R_4 is selected from the group $-N(R_7)R_6O-$, $-N(R_7)R_6N(R_7)-$, OR_6O, $-OR_6N(R_7)-$, where R_6 is alkyl;

R_5 is selected from the group alkyl, aryl, including heteroaryl, $-COR_7$, SO_2R_7, $-C(S)\,NHR_7$, $-C(NH)NHR_7$, $-COR_{10}$,

$-C(O)NHR_7$ and $-N(R_7)\,N{=}C(R_7)(R_{10})$

where R_7 is selected from the group H, alkyl or aryl, including heteroaryl;

R_2 is selected from the group F, Cl, Br, I, OR_7, NO_2, alkyl, aryl, including heteroaryl, formyl, acyl, $C(O)NR_7R_{10}$, $C(O)OR_7$,NR_7R_{10}, $N=C(R_7)(R_{10})$, SR_7, SO_2NH_2, SO_2 alkyl and SO_2aryl,

and m is 0, 1 or 2

R_3 is selected from the group R_7, OR_7, $N(R_7)$ (R_{10}), $N=C(R_7)$ (R_{10}), $N(R_7)$ $N(R_7)$ (R_{10}), $N(R_7)$ $N=C(R_7)$ (R_{10}) and $CH(R_7)C(O)R_8$

where R_8 is selected from the group R_7, OR_7 and NR_7R_{10};

R_9 is selected from the group consisting of hydrogen, alkyl, aryl, including heteroaryl, $C(O)R_{10}$, $-SO_2R_{10}$, $-C(S)NHR_{10}$, $-C(NH)$ NH (R_{10}), $-C(O)$ NHR_{10},

R_{10} is selected for the group H, alkyl or aryl, including heteroaryl and in each case optionally different from substituent R_7

X is selected from group consisting of a halide ion, acetate ion, perchlorate ion, sulfonate ion, oxalate ion, citrate ion, tosylate ion, maleate ion, mesylate ion, carbonate ion, sulfite ion, phosphoric hydrogen ion, phosphonate ion, phosphate ion, BF_4^-, PF_6^-.

with proviso that,

(i) when two alkyl groups are present on the same carbon or nitrogen, they are optionally linked together to form a cyclic structure and

(ii) the nitrogen of heteroaryl ring of R_{10}, when present, is optionally quaternized with compound such as $X\text{-}CH_2C(O)\text{-}R_3$.

2. The compound as claimed in claim 1, wherein $-C(O)R_1$ group is at position 3 or 4.

3. The compound as claimed in claims 1 or 2, wherein the position for $-C(O)R_1$ group is at position 3.

4. The compound as claimed in claims 1, 2 or 3 wherein m is 0 or 1.

5. The compound as claimed in claims 1 or 4, wherein m is 0.

6. The compound as claimed in claims 1 or 2, wherein X is a halide ion.

7. The compound as claimed in claim 1, which is selected from the group consisting of the following compounds:

(a) N,N'-bis[3-carbonyl-1-(2-thien -2'- yl -2-oxoethyl) -3-pyridinium]hydrazine dibromide or a pharmaceutically acceptable salt thereof.

(b) 1-(2-ethoxy -2-oxoethyl) -3-(2-(2-pyridyl)hydrazinocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(c) 1-(2-ethoxy -2-oxoethyl) -3-(2-(benzoyloxy) ethylamino carbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(d)N,N'-bis[3-carbonyl-1-(2-phenyl-2-oxoethyl)pyridinium]hydrazine dibromide or a pharmaceutically acceptable salt thereof.

(e) 1-(2-phenyl-2-oxoethyl)-3-(hydrazinocarbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

(f) 1-(2-thien -2'-yl -2-oxoethyl) -3-(methanesulfonyl hydrazinocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(g)N,N'-bis[3-carbonyl-1-(2-(2,4-dichlorophenyl)-2-oxoethyl)pyridinium] hydrazine dibromide or a pharmaceutically acceptable salt thereof.

(h) 1-(2-phenyl -2-oxoethyl) -3-(methanesulfonyl hydrazinocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(i) 1-(2-ethoxy -2-oxoethyl) -3-(methanesulfonyl hydrazinocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(j) 1-(2-phenyl-2-oxoethyl)-3-(phenylsulfonylhydrazino carbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(k) 1-(2-phenyl-2-oxoethyl)-2-chloro-3-(phenylsulfonyl hydrazino carbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(l) 1-(2-thien -2'-yl -2-oxoethyl)-4-(2-(benzoyloxy) ethyl aminocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(m)1- (2- (2, 4- dichlorophenyl) -2-oxoethyl) -3-(2- (benzoyloxy) ethylaminocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(n) 1-(2-phenyl -2-oxoethyl) -3-(2-(methoxy) carbonyl) ethyloxy carbonyl pyridinium bromide or a pharmaceutically acceptable salt thereof.

(o) 1-(2-ethoxy -2-oxoethyl) -3-(2-(benzoyloxy) ethyloxy carbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

8. A process for the preparation of compounds of the pyridinium series as claimed in claim 1, which comprises preparation of the properly substituted pyridine derivative, according to the desired end products, followed by quaternization of the substituted pyridine derivatives, with appropriate reagent by refluxing in alcoholic solvents and/or high boiling solvents for 6 - 48 hrs. to get the desired compounds.

9. The use of compound of general formula I as defined in claim 1, in the manufacture of a medicament for diabetic complications and aging-related diseases, including kidney disease, nerve damage, retinopathy, atherosclerosis, microangiopathy, endothelial dysfunctions, dermatological conditions, discoloration of teeth and other organ dysfunctions.

10. The use as claimed in claim 9, wherein said compound is selected from the group consisting of :

(a) N,N'-bis[3-carbonyl-1-(2-thien -2'-yl -2-oxoethyl) -3-pyridinium]hydrazine dibromide or a pharmaceutically acceptable salt thereof.

(b) 1-(2-ethoxy -2-oxoethyl) -3-(2-(2-pyridyl)hydrazinocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(c) 1-(2-ethoxy -2-oxoethyl) -3-(2-(benzoyloxy)ethylamino carbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(d) N,N'-bis[3-carbonyl-1-(2-phenyl-2-oxoethyl)pyridinium]hydrazine dibromide or a pharmaceutically acceptable salt thereof.

(e) 1-(2-phenyl-2-oxoethyl)-3-(hydrazinocarbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

(f) 1-(2-thien -2'-yl -2-oxoethyl) -3- (methanesulfonyl hydrazinocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(g)N,N'-bis [3-carbonyl -1- (2-(2,4-dichlorophenyl) -2-oxethyl) pyridinium] hydrazine dibromide or a pharmaceutically acceptable salt thereof.

(h) 1-(2-phenyl -2-oxoethyl) -3-(methanesulfonyl hydrazinocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(i) 1-(2-ethoxy -2-oxoethyl) -3-(methanesulfonyl hydrazinocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(j) 1-(2-phenyl-2-oxoethyl)-3-(phenylsulfonylhydrazino carbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(k) 1-(2-phenyl-2-oxoethyl)-2-chloro-3-(phenylsulfonyl hydrazino carbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(l) 1-(2-thien -2'-yl -2-oxoethyl)-4-(2-(benzoyloxy) ethylaminocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(m) 1-(2-(2,4-dichlorophenyl) -2-oxoethyl) -3-(2-(benzoyloxy)ethylamino carbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

(n) 1-(2-phenyl -2-oxoethyl) -3-(2-(methoxy) carbonyl) ethyloxy carbonyl pyridinium bromide or a pharmaceutically acceptable salt thereof.

(o) 1-(2-ethoxy -2-oxoethyl) -3-(2-(benzoyloxy) ethyloxy carbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

11. A pharmaceutical composition for treatment of diabetic complications and aging related diseases which comprises a pharmaceutically effective amount of one or more compounds of general formula I, as defined in claim 1, or pharmaceutically acceptable salt(s) thereof in admixture with a pharmaceutically acceptable carrier, diluent, solvent or excepient.

12. A pharmaceutical composition as claimed in claim 11, in the form of an oral formulation.

13. A pharmaceutical composition as claimed in claim 12, wherein said pharmaceutically acceptable carrier is selected from one or more of the compounds starch, lactose, polyvinyl pyrolidone (K-30), talc and magnesium stearate.

14. A pharmaceutical composition as claimed in claim 11, in the form of a parenteral formulation.

15. A method for the preparation of a parenteral formulation as claimed in claim 14, wherein the said process comprises dissolving the active ingredient of general formula I, as defined in claim 1, in polyethylene glycol 400 and diluting the solution so obtained, with an isotonic solution or water to the desired concentration.

16. Pharmaceutical composition as claimed in claim 11, in the form of lotions, oral rinse and toothpaste.

17. A method for treating a diabetic patient by breaking the preformed AGE, within said patient, which comprises, administering an effective amount of a compound as claimed in claim 1, either singly, or in combination with other drugs for antidiabetic therapy.

18. A method of preventing or treating diseases caused by diabetes and aging related complications, which comprises, administering to a patient in need thereof, an effective amount of a compound of formula I, as claimed in claim 1, either singly or in combination with a pharmaceutically acceptable carrier, diluent or excepient .

19. The method as claimed in claim 18, wherein the disease prevented or treated is a nephrological disorder, neurological disorder, atherosclerosis, retinal

disorder, dermatological disorder, non-enzymatic browning of oral cavity, endothelial or other organ dysfunction and growth impairment.

INTERNATIONAL SEARCH REPORT

Int· ·ional Application No
PCT/IB 99/01683

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D213/80 C07D213/82 C07D213/87 C07D409/14 C07D409/06
A61K31/4425 A61K31/4436 A61K31/444 A61P37/00 A61P3/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JINDAL, SHASHI ET AL: "Synthesis of possible hypoglycemic compounds. Part V. Synthesis of N-(benzoylmethyl)-3-'hydroxy'alkyl(aryl)!methylaminocarbonyl!pyridinium iodides" INDIAN DRUGS (1995), 32(7), 317-19 , XP000909803 the whole document ---	1-19
X	TIWARI, S. S. ET AL: "Possible antiparkinsonian compounds. VII. Synthesis of amidomethylation products with N-hydroxymethylnicotinamide and their conversion into quaternary ammonium iodides" J. INDIAN CHEM. SOC. (1975), 52(2), 166-7, XP000909760 table 2 --- -/--	1-6, 11-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 June 2000	10. 07. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schmid, J-C

5

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/IB 99/01683

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	MOCANU, G. ET AL: "Macromolecular drug conjugates. IV. Nicotinic acid and nicotinamide/dextran derivatives" S.T.P. PHARMA SCI. (1994), 4 287-91 , XP000909810 page 289; figure 2 ---	1-6, 11-16
X	SAREL, SHALOM ET AL: "Domain-Structured N1,N2-Derivatized Hydrazines as Inhibitors of Ribonucleoside Diphosphate Reductase: Redox-Cycling Considerations" J. MED. CHEM. (1999), 42(2), 242-248 , XP000910109 compounds 2,5 page 246, right-hand column ---	1
X	DEMCHENKO, A. M. ET AL: "Preparation and use of.alpha.-bromomono- and -bromobis'difluoromethoxyacetophenones! in the synthesis of polymethyleneimidazoles with an angular nitrogen atom" CHEM. HETEROCYCL. COMPD. (N. Y.) (1998), VOLUME DATE 1997, 33(10), 1191-1195 , XP000909851 compound XX ---	1
X	CHEMICAL ABSTRACTS, vol. 120, no. 8, 21 February 1994 (1994-02-21) Columbus, Ohio, US; abstract no. 90688, ONODERA, AKIRA ET AL: "Silver halide photographic material containing hydrazine derivative fogging agent" XP002139440 RN 152332-92-8 RN 152333-01-2 RN 152333-08-9 abstract & JP 05 216151 A (KONISHIROKU PHOTO IND, JAPAN) 27 August 1993 (1993-08-27) ---	1
X	PANDEY, V. K. ET AL: "Synthesis of 2,3-substituted-N1-(acetophenone thiosemicarbazone) pyridinium iodides as potential biodynamic agents" INDIAN DRUGS (1983), 20(12), 492-4 , XP000909902 page 492, left-hand column, paragraph 2 ---	1

-/--

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/IB 99/01683

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 96, no. 13, 29 March 1982 (1982-03-29) Columbus, Ohio, US; abstract no. 99045, MAKSIMOVIC, MATEJ ET AL: "The in vitro protective and reactivator effect of bisquaternary derivatives of 4-(hydroxyiminomethyl) pyridine on acetylcholinesterase of erythrocytes inhibited by soman and sarin" XP002139441 RN 78368-92-0 Pyridinium,3-''(2-hydroxyethyl)amino!carbo nyl!-1-'3-'4-'(hydroxyimino)methyl!pyridin io!-2-oxopropyl!-, dibromide RN 78368-93-1 Pyridinium,4-''(2-hydroxyethyl)amino!carbo nyl!-1-'3-'4-(hydroxyimino)methyl!pyridini o!-2-oxopropyl!-,dibromide abstract & NAUCNO-TEH. PREGL. (1981), 31(3), 24-8 , ---	1,11-16
X	BINENFELD, ZLATKO ET AL: "Reactivating effects of pyridinium salts on acetylcholinesterase inhibite by organophosphorus compounds" ACTA PHARM. JUGOSL. (1981), 31(1), 5-15 , XP000909901 compounds 26,27 table III ---	1,11-16
X	CHEMICAL ABSTRACTS, vol. 65, no. 6, 12 September 1966 (1966-09-12) Columbus, Ohio, US; abstract no. 8891f, ERGENC, NEDIME: "condensation product of chloromethyl antipyrinyl ketone and isoniazid" XP002139442 RN 6794-28-1 Pyridinium,1-'(antipyrinylcarbonyl)methyl! -4-carboxy-, chloride, hydrazide abstract & ISTANBUL. UNIV. ECZACILIK FAK. MECMUASI, vol. 1, no. 1, - 1965 pages 82-9, --- -/--	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int· ·ional Application No
PCT/IB 99/01683

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 52, no. 3, 1958 Columbus, Ohio, US; abstract no. 12860f, KAO, I-SHENG ET AL: "prepn. and the antituberculostatic action of chloroacetyl derivs. of isonicotinoylhydrazine and derivs. of 2,6-diaminoisonicotinic acid" XP002139443 RN - 114302-80-6 4-Carboxy-1-(carboxymethyl)pyridinium chloride, 4-(2-chloroacetylhydrazide) abstract & HUA HSÜEH HSÜEH PAO, vol. 22, - 1956 pages 566-71,	1,11-16
X	GB 822 351 A (CILAG-CHEMIE LTD.) claim 1; example 11	1
A	US 5 853 703 A (EGAN JOHN J ET AL) 29 December 1998 (1998-12-29) cited in the application the whole document	1-19

5

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/IB 99/01683

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 17-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/IB 99/01683

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 5216151 A		NONE	
GB 822351 A		CH 335521 A	
US 5853703 A	29-12-1998	US 5656261 A	12-08-1997
		AU 714607 B	06-01-2000
		AU 4759996 A	07-08-1996
		BR 9607598 A	30-11-1999
		CA 2210684 A	25-07-1996
		CN 1185736 A	24-06-1998
		EP 0808163 A	26-11-1997
		FI 973031 A	15-09-1997
		JP 10512864 T	08-12-1998
		NO 973308 A	18-09-1997
		WO 9622095 A	25-07-1996
		US 6007865 A	28-12-1999